EFFECT OF A BRAZILIAN EUPHORBIACEAE ON THE PENIAL COMPLEX OF *BIOMPHALARIA GLABRATA*

M. Z. ROUQUAYROL[1], E. A. MALEK[2], A. A. CRAVEIRO[3],
M. Z. B. BEZERRA[3] AND M. I. L. MACHADO[3]
[1]UNIVERSIDADE DE FORTALEZA, FORTALEZA, CEARA AND CNPq BRAZIL
[2]DEPARTMENT OF TROPICAL MEDICINE, TULANE MEDICAL CENTER,
NEW ORLEANS, LOUISIANA 70112, U.S.A.
[3]DEPARTMENT OF ORGANIC CHEMISTRY, UNIVERSIDADE FEDERAL DO CEARA,
FORTALEZA, CEARA, BRAZIL

ABSTRACT

Several materials prepared from the leaves of "Canela de Urubu" *Croton sp.*, a natural plant in the northeast of Brazil, kill *Biomphalaria* spp. intermediate hosts of *Schistosoma mansoni*, at 250 to 1000 p.p.m. However, lower concentrations are not lethal to the snails, but cause relaxation of these animals and eversion of the penial complex. As is the case in the process of copulation, the preputium is everted and protrudes outside the snail. Then the anterior tip of the relaxed penis protrudes outside the preputium.

A total of 33 out of 113 (29.2%) individuals of *B. glabrata* and 2 out of 70 (3%) of *B. straminea* showed eversion of the penial complex when subjected to various concentrations of the hydrolate. The best results were obtained with 70 p.p.m. of the prepurified fraction, 50 p.p.m. of the hydrogenated, and 74 p.p.m. of the pure substance. Using extracts from "Canela de Urubu" seems to be a good method to demonstrate the detailed morphology of the penial complex of certain species of *Biomphalaria*, and to anesthetize the snails for internal surgery and physiological studies.

"Canela de urubu", *Croton* sp-09., also known as "Ervanço" (Craveiro et al., 1981) is one of the common natural plants in the northeast of Brazil. Its molluscicidal properties have been demonstrated against the snail *Biomphalaria glabrata,* intermediate host of *Schistosoma mansoni* in many parts of Brazil, (Rouquayrol et al., in preparation). It was shown in the latter studies that several materials prepared from the leaves of this plant killed snails at certain concentrations as follows: the pure hydrolate in 24 hours; the hydroalcoholic extract at 1000 ppm in 24 hours; the prepurified fraction from hydrolate at 250 and 500 ppm in 24 hours and one pure substance, geijereno isolated from the essential oil at 25, 50 and 100 ppm at 8 hours.

During the molluscicidal study another property of the plant became evident and that is lower concentrations of the hydrolate and the prepurified fraction are not lethal to the snails, in general, but cause eversion of the snail's penial complex. This property of the plant was investigated further, and the results are included in this paper.

MATERIALS AND METHODS

The plant "Canela de urubu", *Croton* sp-09 was collected from Aracati, State of Ceara, Brazil, in plastic sacs that were transported to the Department of Organic Chemistry at Federal University of Ceara (UFC) in refrigerated cars (5–10°C) to prevent evaporation. Extractions of materials from plants were carried out in the laboratory of Organic Chemistry by several procedures. 1) Hydrolate: Extractions were made with steam distillation of 3.5 kg of leaves in an extractor developed in the UFC laboratories (Craveiro et al. 1976). The mixture of vapors is condensed to a liquid state, water and essential oil are separated in different containers. The water so obtained is called a hydrolate whose constituent substances and concentration are not known. 2) Hydroalcoholic extract: Finely ground, plant material (10 g) was boiled with 50% ethanol (200 ml) with stirring for 10 minutes. The mixture was filtered, the residue discarded and the filtrate was used for tests. 3) Prepurified fraction: Extraction of the hydrolate (12 1) with ether (9 × 300 ml) afforded, after evaporation of the solvent, a residue (2 g) that was used directly for experiments with snails. 4) Pure substance: Fractionation of the above residue using column chromatography on silica gel gave a liquid substance (456 mg) with sesquiterpene skeleton and ketone function. 5) Hydrogenation: The above substance (200 mg) was hydrogenated with Pt/C 10% in acetic acid/methanol (8:2) (2 ml) in hydrogen atmosphere for 12 hours.

American Malacological Bulletin, Vol. 1(1983):67–70

The snails used in the experiments were *Biomphalaria glabrata* originally from Paulista, Pernambuco (snails 5–10 mm in diameter); *Biomphalaria straminea* from Redensao and Pentecoste, state of Ceara; *Biomphalaria tenagophila,* originally from Belo Horizonte, State of Minas Gerais and *Lymnaea (Stagnicola) elodes* from Michigan, U.S.A.

The procedures used for testing the materials were as follows:

1) Hydrolate: Dilutions of 1:1, 1:2, 1:5, 1:10 and 1:20 were made by addition of dechlorinated tap water. Observations of 2–5 snails per container were made by the naked eye and stereo microscope at 2 to 72 hours, and number of snails with everted penial complex and number of survivors were recorded.

2) Prepurified fraction GM-10 (f-56.69): One ml of absolute ethanol was added to 50 mg of the fraction to make it soluble and addition of 99 ml of water resulted in a concentration of 500 ppm. From this stock solution, other concentrations were prepared.

3) Hydrogenated: One ml of absolute ethanol was added to 20 mg of the formulation to make it soluble, and addition of 99 ml of water resulted in a concentration of 200 p.p.m. From this stock solution, other concentrations were prepared.

4) Pure substance (T.I. 7635; f-699 f-15): One ml of absolute ethanol was added to 39.5 mg of the substance to make it soluble, and addition of 99 ml of water resulted in a concentration of 395 p.p.m. From this stock solution other concentrations were prepared.

In the case of all the materials used, counts were made during 24 hours of the heart beat of *B. glabrata.*

Most of the experiments were carried out at UFC, and confirmatory tests were also conducted in New Orleans, using the same species of snails which were mailed from Fortaleza, in addition to other species.

Longitudinal serial histological sections were prepared from paraffin blocks, of the normal and of the everted penial complex. These sections were stained with hematoxylyn and eosin.

RESULTS

The effect of "Canela de Urubu" on the penial complex was more evident on *B. glabrata* than on *B. straminea* and there was no effect on *B. tenagophila. Lymnaea (Stagnicola) elodes*, like *B. tenagophila,* showed negative results.

The commencement of eversion varied from one snail to the other, but in some snails eversion started only two hours after contact with the hydrolate.

A total of 33 out of 113 (29.2%) of *B. glabrata* and 2 out of 70 (3%) of *B. straminea* showed eversion of the penial complex, using the hydrolate. With all concentrations of the hydrolate used there were 174 survivors out of 205 or 85% of *Biomphalaria* spp. With the prepurified fraction the best results for eversion were obtained with 70 p.p.m., with two out of five snails showing eversion. With the hydrogenated the best results were obtained with 50 p.p.m., with two out of five snails exhibiting eversion. It should be noted that at 74 p.p.m. the pure substance caused eversion of the penial complex of 71.4% of *B. glabrata.* The pure substance isolated from "Canela de Urubu" is probably a new substance, a sesquiterpene, whose final structure is under investigation in our laboratories.

In its inverted normal position the penial complex of *Biomphalaria* spp. consists of the preputium, which is a cylindrical and elongated organ and opens to the outside at the male genital opening situated near the base of the left tentacle. At the other end, the preputium continues as the vergic sac which contains the verge. The vergic sac is much smaller in diameter than the preputium. The preputium, in various species, varies in length compared to that of the vergic sac, but in *Biomphalaria glabrata* they are of almost equal length. Internally the preputium has two longitudinal muscular columns, the pilasters. Separating the cavity of the preputium from that of the vergic sac there is a muscular and secretory diaphragm consisting of several folds.

The gross morphology and histological details of the penial complex of a related species *Biomphalaria alexandrina* (= *B. boissyi*) were reported upon by Malek (1954). The internal muscular elements, and the blood (hemolymph) spaces of both preputium and the vergic sac were described and illustrated in detail. The gross morphology of the penial complex of *B. glabrata, B. straminea* and *B. tenagophila* was treated by Barbosa et al. (1968) and Paraense and Deslandes (1956).

The effect of certain concentrations of materials extracted from "Canela de Urubu" was the eversion of the penial complex of many specimens of *Biomphalaria glabrata* and a few of *B. straminea.* At the start of eversion, as is also the case in the process of copulation, the preputium is everted and protrudes outside the snail, that is, outside the male genital opening (Fig. 1). The inside layers of the preputium are now on the outside, and the outer surface of this organ is inside. The diaphragm is now at the tip of the everted preputium and its folds appear in the form of a rosette (Fig. 2).

The eversion of the preputium is followed by protrusion of the vergic sac inside the preputium. The penis is erected further, and its anterior tip shows protruding outside the preputium, usually an hour or two after eversion of the preputium. Serial longitudinal sections of the everted preputium showed the muscular pilasters on the outside, and the surface epithelium on the inside. Between the two layers there is a spaceous connective tissue region, with a few muscle fibers, and with abundant hemolymph spaces.

During relaxation of the snail, that is, with the head-foot region extending outside the shell, eversion of the preputium and erection of the penis continued for up to three days in the "Canela de Urubu" materials. When such snails were returned to water the penial complex retracted inside the animal and they returned to moving normally.

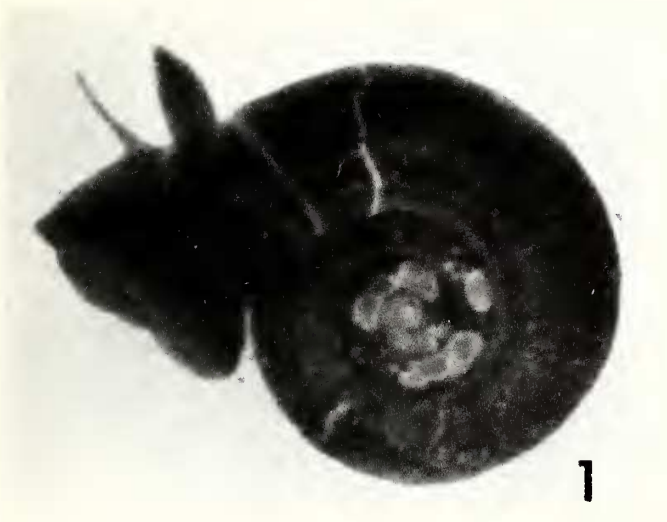

Fig. 1. *Biomphalaria glabrata* with preputium everted and protruded outside the snail next to the tentacle. Horizontal field width = 2.2 cm.

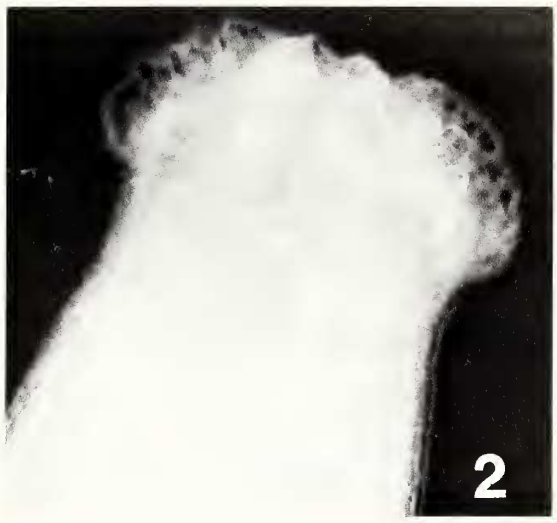

Fig. 2. Everted preputium enlarged showing folds of the diaphragm appearing in the form of a rosette. Horizontal field width = 6.0 mm.

Our observations on the effect of "Canela de Urubu" on the heart beat showed that although with some snails there was a noticeable reduction, such a phenomenon was not consistent, and it seems that more experiments and observations are necessary to clarify this matter.

DISCUSSION

The present study showed that various materials extracted from "Canela de urubu" caused relaxation of the snail, eversion of the preputium and protrusion of the penis of a large number of *Biomphalaria glabrata* and a few *B. straminea*. This condition of the penial complex normally takes place in preparation for copulation in these hermaphroditic snails. That the snails usually survived for up to three days in a relaxed condition and recovered and returned to normal when placed back in water indicates that its effect is similar to that of some substances that are used for anesthesia of snails, rather than the effect of narcotics. Its effect is, however, similar to that of some narcotics of snails such as menthol, in that it causes eversion of the penial complex. It has been our experience that when menthol is used as a narcotic to relax snails before fixation for morphological studies, the snails will have, in addition to relaxation, various degrees of eversion of the penial complex.

We can foresee several uses for "Canela de Urubu". It is a good method to demonstrate the detailed morphology of the penial complex of species of *Biomphalaria*. Further studies may prove its use as an anesthetic for internal surgery on snails, and physiological studies, for example, on the nervous system, muscles and heart. It can also be used as a tool in crossing experiments among hermaphroditic snails. This can be affected by cutting the penis after its relaxation and protrusion, and experimenting with the resulting snail as a female snail.

LITERATURE CITED

Barbosa, F. S., Berry, E. G., Harry, H. W., Hubendick, B., Malek, E. A. and Paraense, W. L. 1968. *A Guide for the Identification of the Snail Intermediate hosts of Schistosomiasis in the Americas*. Pan American Health Organization, Scientific Publication No. 168, 122 pp. Washington, D.C.

Craveiro, A. A., Fernandes, A. G., Andrade, C. H. S., Matos, P. J. A., Alencar, J. W. and Machado, M. I. L. 1981. *Oleos Essenciais de Plantas do Nordeste*, ed. Universidade Federal do Ceara, Fortaleza, Brasil, 210 pp.

Craveiro, A. A., Matos, F. J. A. and Alencar, J. W. 1976. A simple and inexpensive steam generator for essential oils extraction. *Journal of Chemical Education* 53:652.

Malek, E. A. 1954. Morphological studies on the family Planorbidae (Mollusca: Pulmonata). II. The genital organs of *Biomphalaria boissyi* (Subfamily Planorbinae H. A. Pilsbry, 1934). *Transactions of the American Microscopical Society* 73:285–296.

Paraense, L. W. and Deslandes, N. 1956. Diagnostic characters of the Brazilian species of *"Australorbis"* (Pulmonata: Planorbidae). *Revista Brasileira Biologia* 16:281–286.